

# Assessment of transport and isolation methods for gonococci

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**SUMMARY** Urethral discharge from men was diluted to give heavy and light inocula and cultured on seven different solid culture media, including two transport/isolation media, or held in three types of semi-solid transport medium for varying periods and then cultured. The amount of growth was quantitated and the performance of the different systems compared. Fresh non-selective media were best, with up to two failures in 254 cultures on each medium. With selective media there were 9-23 failures with heavy inocula and 22-47 failures with dilute inocula. For Transgrow or the Jembec system incubation before holding at ambient temperature was better than holding followed by incubation. Transport media yielded good results if cultures were set up within six hours; only minor losses occurred after 24 hours.

## Introduction

Many methods have been suggested for the transport of samples from patients suspected of having gonorrhoea and for the primary isolation of *Neisseria gonorrhoeae*. Various transport media, isolation media inoculated in the clinic, and more recently media for the combined transport and growth of the organism have all been used. We have carried out an assessment of some of these methods.

## Materials and methods

### URETHRAL SAMPLES

Samples of urethral discharge containing Gram-negative intracellular diplococci, which was sufficiently profuse, were collected both for routine diagnosis and for this study from men attending the department of genitourinary medicine at St Thomas's Hospital, London.

### MEDIA

Seven preparations of solid culture media, three solid transport/isolation media, and three types of semi-solid transport medium were assessed.

The solid media (A-G) were either supplied ready-poured in divided plates by Difco Ltd or Oxoid Ltd or were prepared in St Thomas's Hospital (table 1).

The formulae of these media were as follows:

*GC non-selective* (A) (Difco)—proteose peptone No 3 15 g/l, corn starch 1 g/l, dipotassium phosphate 4 g/l, monopotassium phosphate 1 g/l, sodium chloride 5 g/l, agar 15 g/l, haemoglobin 10 g/l, dextrose 1 g/l, and supplement B 10 ml/l;

*GC selective* (B) (Difco)—as above, plus vancomycin 3 mg/l, colistin sulphomethate 7.5 mg/l, nystatin 12 500 units/l, and trimethoprim 5 mg/l;

*Transgrow*<sup>1</sup> (C) (Difco)—as GC selective, but with agar 20 g/l, supplement B 20 ml/l;

*VCNT*<sup>2</sup> (D, E, and F)—heart infusion agar (Difco 0044-01), saponin-lysed horse blood 10% (heated blood was used for the medium in Jembec plates), plus antibiotics as above; and

*Lysed blood agar* (G) (St Thomas's Hospital)—DST agar (Oxoid CM 261); saponin-lysed horse blood.

The three solid transport/isolation systems provided by Oxoid were:

*Transgrow* in bottles;

*VCNT* in Jembec plates<sup>3</sup>; and

*Chocolate agar VCNT* in Jembec plates.

The semi-solid transport media were:

*Stuart's transport medium* (Southern Group Laboratories)—sodium glycerophosphate 10 g/l, thioglycollic acid 1 g/l, calcium chloride 1 g/l, methylene blue 0.02 g/l, and agar (Becton Dickson) 2.2 g/l;

*Stuart's transport medium* (Oxoid R22)—sodium glycerophosphate 10 g/l, sodium thioglycollate 0.5 g/l, cysteine hydrochloride 0.5 g/l, calcium chloride 0.1 g/l, methylene blue 0.001 g/l, and agar No 1 (Oxoid L11) 10 g/l; and

*Amies's transport medium* (Oxoid 425)—sodium

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Received for publication 31 December 1980

TABLE 1 *Bacterial counts from small inocula on seven different media\**

	<i>Transgrow</i> (C)	<i>GC selective</i> (B)	<i>VCNT</i> (D)	<i>VCNT</i> (E)	<i>VCNT</i> (F)	<i>Lysed-blood</i> <i>agar</i> (G)	<i>GC non-</i> <i>selective</i> (A)
Container	Bottle	Petri dish	Petri dish	Jembec	Petri dish	Petri dish	Petri dish
Poured by	Difco	Difco	Oxoid	Oxoid	St Thomas's	St Thomas's	Difco
No of observations	253	250	254	114	254	243	245
Mean No of colonies/plate	25	27	30	31	32	40	41

\* In order of increasing efficiency

chloride 8 g/l, potassium chloride 0.2 g/l, calcium chloride 0.1 g/l, magnesium chloride 0.1 g/l, mono-potassium phosphate 0.2 g/l, disodium phosphate 1.5 g/l, sodium thioglycollate 1.0 g/l, charcoal 10 g/l, and agar 3.6 g/l.

#### EXPERIMENT 1

The solid media for isolation of *N gonorrhoeae* were compared in the first experiment.

The sample of urethral pus was collected on a plain swab, which was broken off into 1 ml of nutrient broth containing 10% lysed horse blood, and brought to the laboratory within two hours. When the sample was received in the laboratory the swab was discarded after the liquid had been expressed from it. The broth and a  $10^{-3}$  dilution of it were used in standard loopfuls of 0.007 ml to inoculate the various types of solid medium listed in table I in parallel. Cultures were then incubated overnight at 36°C in 10% CO<sub>2</sub>, except for the Transgrow bottle, which already contained CO<sub>2</sub>, and the Jembec plate, which included a tablet from which CO<sub>2</sub> was generated.

The growth from a heavy inoculum was roughly quantitated but the individual colonies that grew from light inocula were counted whenever possible. Total counts for light inocula of each sample were compared by paired *t*-tests provided at least one plate had more than 10 colonies in the inoculum and provided none had more than 200 colonies. An analysis of variance was also performed on the counts from light inocula. The various patterns of complete failure of isolation were enumerated for cultures from heavy and light inocula.

#### EXPERIMENT 2

The solid combined transport and culture media were compared in the second experiment.

Three solid media, Transgrow and VCNT with lysed or heated blood in Jembec plates, were inoculated with heavy inocula from lysed-blood broth as in the first experiment and then either left at room temperature for periods of up to two days before incubation or incubated overnight and then left for periods of up to four days before subculture.

#### EXPERIMENT 3

The semi-solid transport media were compared in the third experiment.

Six swabs were soaked in the heavy lysed-blood broth suspension of gonococci prepared for the first experiment and placed in pairs of each of the three types of semi-solid transport medium. One of each pair was held at 4°C and the other at room temperature. The swab was used to provide subcultures to warm lysed-blood agar at daily intervals for five days. It was shown in a preliminary experiment that repeated use of the same swab did not significantly deplete it of gonococci.

#### ORGANISMS

Gonococci were identified as in previous investigations by Gram-staining, the oxidase test, and specific immunofluorescence. Sugar fermentation tests were also performed for the few organisms that did not fluoresce.

#### Results

##### SOLID MEDIA

The results of *N gonorrhoeae* counts on the various media for 254 parallel cultures from dilute inocula in experiment 1 are shown in table I. Results of a further 70 cultures were excluded because the number of colonies growing from the dilute inoculum did not exceed 10 on any medium; those of a further six cultures were also excluded because counts were too high. The total number of observations on each medium varied because plates were occasionally contaminated or unavailable—the latter especially with medium E (table I). On the basis of paired *t*-tests<sup>4</sup> the two non-selective media, A (GC non-selective) and G (lysed-blood agar), did not differ from each other, but there was a significant difference between these two and the rest and between media C (Transgrow) and F (St Thomas's VCNT).

With low inocula 159 (63%) cultures grew the organism on all plates. Failures were commonest on selective media B (47), D (40), C (25), and F (22), were uncommon on lysed-blood agar (G) (2), and did

TABLE II *Viability of gonococci in three transport-culture systems*

Medium for transport/culture	Incubation	% Failure from cultures or subcultures after:					No of tests
		6 hrs	24 hrs	2 days	3 days	4 days	
Transgrow	Delayed	2.4	5.7	33.6	NT	NT	122
	Immediate	0	1.4	4.0	8.8	24.3	148
VCNT in Jembec plate	Delayed	2	26	86	NT	NT	50
	Immediate	0	2	16	70	92	60
Chocolate VCNT in Jembec plate	Delayed	0	14	68	NT	NT	50
	Immediate	0	4	16.7	26.2	81	81

NT = not tested

not occur with medium A. Results for medium E came from only 84 cultures, and a corrected failure rate for 254 cultures would be 24, making the medium similar to C and F.

When the larger inocula were considered in the same way 319 of 334 cultures gave assessable results. In 88% gonococci were isolated from all media. Failures were commonest on selective media B (23 failures), D (19), C (15), E (6, or 11 after correction), and F (9), and were very uncommon on the non-selective media A (2) and G (1).

#### TRANSPORT/CULTURE MEDIA

The results of tests for the viability of gonococci on the three solid transport culture media (experiment 2) either kept for up to two days before incubation (delayed) or first incubated (immediate) and then left at room temperature for up to four days before subculture are shown in table II. In the "delayed" series, in most instances incubation within six hours yielded gonococci but incubation after 24 hours resulted in a 5.7% loss of isolates from Transgrow in bottles, and 14% from chocolate agar, and 26% from lysed-blood agar in Jembec plates; after two days these figures were 33.6%, 68%, and 86% respectively.

We noted a marked seasonal variation in results for media held at room temperature before incubation. During the period January to March failure rates for Transgrow were 4% after six hours, 10% after 24 hours, and 54% after 48 hours. During April to June the respective figures were 2%, 4%, and 26%, and in the hot months of July and August (room temperature reaching 35.5°C) there were no losses among 22 cultures kept for up to 48 hours

before incubation. For Jembec plates the figures were 2% after six hours, 26% after 24 hours, and 86% after 48 hours for January to March, and 0%, 14%, and 68% respectively for April to June.

In the "immediate" series, in which cultures were incubated overnight and then examined, sub-cultures within six hours all yielded viable gonococci. A delay of 24 hours before subculture resulted in some loss of viability, and this increased over the four days of the study. In the Transgrow system only a quarter of the cultures were not viable after four days at room temperature whereas in the other two systems most of the cultures had died by this stage.

#### SEMI-SOLID TRANSPORT MEDIA

The results for experiment 3 on semi-solid transport media are shown in table III. Overall, Amies's medium was best, and the modification of Stuart's medium containing more agar was worst. Storage at 4°C was more efficient for Stuart's media but not for Amies's medium. Even with the best system used, a quarter of the cultures were dead after two days and 60% after four days.

#### Discussion

The first experiment was an investigation of combined transport and culture media under good laboratory conditions. We showed, not surprisingly, that for cultures from men non-selective media are best. This superiority was demonstrated by our finding significantly higher mean colony counts on non-selective media than on any other antibiotic-containing media and higher isolation rates. With

TABLE III *Viability of gonococci in three semi-solid transport systems*

Transport medium	Temperature of storage	% Failure from cultures or subcultures after:					No of tests
		6 hrs	24 hrs	48 hrs	72 hrs	96 hrs	
Stuart's agar 1% (Oxoid R22)	Room	0	79	97	100	100	58
	4°C	0	41	95.5	100	100	22
Stuart's agar 0.22% (Southern Group)	Room	0	41	77.3	95.5	100	22
	4°C	0	4.5	36.4	63.6	91	22
Amies's (Oxoid)	Room	0	5.2	24	36.2	60.3	58
	4°C	0	13.7	32	41	59.1	22

heavy or light inocula there were only two or three complete failures on the non-selective media.

There was no clear difference for dilute inocula between the various selective media, the mean colony count ranging from 25 to 32. In terms of outright failure, Difco GC selective medium (B) and Oxoid VCNT (D) fared worst with 47 and 40 failures respectively from dilute inocula; Difco Transgrow (C), Oxoid VCNT with chocolate agar in Jembec plates (E), and St Thomas's Hospital VCNT (F) fared better and approximately equally with 25, 24 (a corrected figure), and 22 failures respectively. Results from heavy inocula were better, but failures—although less common—were seen more often with media B and D than with C, E, and F. It would seem that the use of dilute inocula did in fact constitute a more severe test of the media.

We feel that our overall results support our practice of using selective media for the isolation of *N gonorrhoeae*, even from men (from whom inocula are usually large), and that difficulties in quality control as applied to the commercial products probably accounted for the minor differences that we observed. Clearly, some kind of quality control of media for the isolation of gonococci is essential in the laboratory in which they are used. The most sensitive test in our experience is of their ability to support the growth of small inocula of *N gonorrhoeae* in a series of tests, but this is unfortunately impossible in most laboratories. Growth from the natural inoculum from urethral samples from men with positive results by Gram staining is a practicable alternative.

The second part of the study attempted to simulate delays in combined transport-isolation media reaching the laboratory, as for example by post, and the effect of incubation before or after such a delay. For all our systems, incubation before dispatch was clearly better. This is in contrast to Brown<sup>5</sup>, who found that it made little difference with his modified Transgrow. The Transgrow system was better than the Jembec system with the media that we used; for example, two days after primary incubation only 4% of the Transgrow cultures were dead. We emphasise that, unlike Jephcott *et al.*<sup>3</sup> we did not use Transgrow in Jembec plates.

If cultures were held before incubation there was a striking loss of viability. Even in the Transgrow system one third of the cultures were dead by the second day. Jephcott *et al.*<sup>6</sup> did not detect such deficiencies but studied Transgrow only under conditions of no delay. Chapel and his colleagues<sup>7</sup> held Transgrow inoculated with clinical samples at room temperature for 48 hours before incubation and found that about a quarter of the cultures giving positive results on immediate inoculation on Thayer-Martin medium failed on Transgrow, a result similar

to ours. We conclude that although these transport-culture media performed well if incubated immediately and examined immediately, performance was not nearly so good under conditions that mimic more closely those actually obtaining in most clinics.

The final experiment compared three semi-solid transport media. All three were good, provided cultures were set up from them within six hours. By 24 hours failure was the rule with a modified Stuart's medium containing more agar, although in normal Stuart's and in Amies's media there were only 4-5% failures, depending on the temperature at which the transport media had been held. We are unable to explain our finding that storage at 4°C was better than at room temperature for Stuart's medium but worse for Amies's medium.

Our results draw attention to the problem of delay in setting up or incubating cultures for *N gonorrhoeae*. None of the transport methods fully compensates for delay but for the ones that we studied a delay of 24 hours had least effect on Transgrow incubated and then sent to the laboratory. Slightly inferior results were obtained with Jembec plates incubated and then sent to the laboratory, with Stuart's medium held at 4°C, and with Amies's medium held at room temperature.

We thank Dr C S Nicol and his staff for their participation in this study, which was made possible by a DHSS grant. We also thank Mr M Stoate and Dr S E Smith for their help with statistical analyses.

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